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## Differential involvement of mu-opioid receptors in the rostral versus caudal nucleus accumbens in the reinforcing effects of heroin in rats: evidence from focal injections of $\beta$ -funaltrexamine

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**Abstract** *Rationale:* The nucleus accumbens is a diverse and heterogeneous structure along its rostrocaudal axis. The influence of specific subpopulations of mu-opioid receptors within the NAcc in heroin self-administration has not been documented. *Objectives:* This study was undertaken to investigate the involvement of subregions of the NAcc in heroin self-administration in rats. *Methods:* Male rats were trained to self-administer heroin and then given  $\beta$ -FNA, an irreversible mu-opioid receptor antagonist, into either the rostral or caudal portion of the NAcc. *Results:*  $\beta$ -FNA (0.25–2.5 nmol) attenuated heroin self-administration in a dose-responsive manner when given into the caudal but not rostral NAcc. The number of infusions of 18  $\mu$ g of heroin self-administered was increased by 50–100%. This effect persisted for up to 17 days following administration of the highest dose. These doses of  $\beta$ -FNA were found to decrease [ $^3$ H]DAMGO binding in a dose-responsive manner and the effect was confined to the NAcc, as nearby structures such as the caudate putamen and olfactory tubercles were unaffected. The effect of  $\beta$ -FNA (2.5 nmol) administration into the caudal NAcc was also assessed on the dose-effect curve for heroin. This dose apparently shifted the dose-effect curve to the right initially, followed by an apparent upward shift for up to 17 days after  $\beta$ -FNA administration. *Conclusions:* The caudal portion of the NAcc and its output sites merit further investigation regarding the reinforcing effects of heroin.

**Keywords** Opioid · Nucleus accumbens · Reinforcement · Heroin · Narcotic antagonist · Self-administration

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### Introduction

The nucleus accumbens (NAcc) is a complex structure that has received much attention regarding its role in drug reinforcement. Based upon histological and neurochemical studies, this structure has been divided into three subdivisions: the rostral pole, the shell and the core (Heimer et al. 1995; Zahm 2000). Each substructure possesses distinct afferent and efferent connections, with the rostral pole having some of the characteristics of both the core and shell. Comparisons between the anatomical features of the substructures of the NAcc have been reviewed (Zahm and Heimer 1993). The lateral aspect of the rostral pole of the NAcc possesses similar characteristics as the core regarding substance P and calbindin immunoreactivity, as well as profuse projections to basal ganglia structures. The medial rostral pole and shell of the NAcc, conversely, are characterized by light staining for substance P and calbindin and send projections predominantly to limbic structures. Despite similarities between the lateral and medial aspects of the rostral pole and the core and shell, respectively, sufficient differences exist to suggest that these regions represent three distinct neuroanatomical subsets of the NAcc that probably subserve different functions.

Pharmacologically, most of the attention afforded the NAcc has been focused on distinguishing the role of the core and shell. Opioids interact with their receptors in a variety of input and output regions of both the core and shell of the NAcc. Within the NAcc, mu-opioid receptors are more dense in the rostral pole and shell compared to the core (Tempel and Zukin 1987). Injection of opioid antagonists into the NAcc attenuates the reinforcing effects of heroin in rats; however, the involvement of opioid receptors in specific subregions has not been documented (Vaccarino et al. 1985; Corrigan and Vaccarino 1988). Published injection sites indicate that predominantly caudal sites were examined in previous studies (Vaccarino et al. 1985). Numerous other investigators have found that direct injection of opioids into the accumbens will produce a reinforcing stimulus; however,

these effects have not been examined along the rostrocaudal axis or within specific subregions of the nucleus accumbens (Olds 1982; Goeders et al. 1984).

Several lines of evidence indicate that the NAcc displays a rostrocaudal organization with functional implications in addition to the more widely studied shell and core distinction. Expression of glutamic acid decarboxylase and preproenkephalin within the core of the NAcc follows a rostrocaudal gradient, with expression being higher in the more rostral regions (Rogard et al. 1993). Conversely, there is an increasing rostrocaudal gradient of dopaminergic nerve terminals and dopamine D<sub>1</sub> receptors within the NAcc (Allin et al. 1989). Dopaminergic projection neurons from the ventral tegmental area (VTA) to the caudal, but not rostral NAcc co-release cholecystokinin (CCK) (Hokfelt et al. 1980). Injection of amphetamine into the caudal but not rostral NAcc decreases the threshold for intra-VTA self-stimulation (Ranaldi and Beninger 1994). Repeated nicotine administration selectively increases preproenkephalin mRNA levels in the rostral pole and anterior one-third of the NAcc core, without influencing expression elsewhere in this structure (Mathieu et al. 1996). Therefore, the NAcc appears to be heterogeneous along the rostrocaudal axis with respect to both neurochemical and pharmacological indices.

Laboratory investigations of heroin's reinforcing effects using self-administration paradigms have identified two major circuits involved: a dopamine-dependent circuit that projects to the NAcc from the ventral tegmental area (VTA), and a dopamine-independent circuit that projects from the NAcc to the ventral pallidum (Koob 1992). Depletion of dopamine in the NAcc using 6-hydroxy-dopamine has little to no effect on heroin self-administration (Pettit et al. 1984; Gerrits and Van Ree 1996). Microdialysis studies during heroin self-administration have yielded equivocal findings regarding modulation of extracellular dopamine levels. Dopamine was found to be increased in the rostral pole of the accumbens (Wise et al. 1995), whereas no such action was apparent in more caudal regions of this structure during heroin self-administration (Hemby et al. 1995). Therefore, there is some question regarding the role of subregions of the NAcc in heroin self-administration, particularly with respect to rostral compared to caudal subregions.

These studies were undertaken to determine if differences exist for the involvement of mu-opioid receptors within the nucleus accumbens in heroin self-administration along the rostrocaudal axis. For this purpose,  $\beta$ -funtrexamine, an irreversible mu-opioid antagonist, was injected into either the rostral pole or caudal region of the NAcc in rats trained to self-administer infusions of heroin. The effect of  $\beta$ -FNA on mu- and delta-opioid receptor binding within the nucleus accumbens was evaluated using quantitative receptor autoradiography. These studies will hopefully provide insights into the mechanisms contributing to heroin self-administration in this complex structure.

## Materials and methods

### Subjects

Subjects consisted of 84 male, Fisher 344 rats (Harlan Laboratories, Indianapolis, Ind., USA) weighing 250–350 g at the beginning of the experiment. Animals were kept on a reversed light:dark cycle (dark 0500–1700 hours) in a temperature-controlled environment and kept at 85% of their free-feeding body weight. Water was available ad libitum except during experimental sessions.

### Surgical procedures

All surgical procedures have been described previously (Martin et al. 1995). Briefly, animals were anesthetized with an IP injection of 50 mg/kg pentobarbital and 10 mg/kg atropine methyl nitrate. Chronic indwelling catheters were implanted into the right external jugular vein and extended to the right auricle. The catheter exited the animal between the scapulae and continued through a spring leash, terminating at a fluid swivel (Weeks 1962). The leash was attached at the back of the animal to an implanted polypropylene plate encased in Teflon mesh. Stainless steel guide cannulae (Plastics One, Roanoke, Va., USA) were implanted bilaterally using a Stellar stereotaxic apparatus that automatically corrects for angular placement. Guide cannulae were placed at the dorsal surface of either the rostral (9.0 mm rostral from lambda,  $\pm 1.5$  mm lateral from midline and 5.8 mm ventral from the skull surface) or caudal (7.5 mm rostral from lambda,  $\pm 1.5$  mm lateral from midline and 5.8 mm ventral from the skull surface) nucleus accumbens. Cannulae were angled 10° lateral from vertical to allow for clearance of dummy cannulae and the head was placed at a slight downward angle (incisor bar set at  $-2.5$  mm). The cannulae were secured by dental acrylic and stainless steel, self-tapping screws. Animals were administered 75,000 units IM of penicillin G procaine (Butler Co., Columbus, Ohio, USA) and all exterior surgical wounds were dressed with antibiotic powder (Polysporin; Wellcome-Glaxo, Research Triangle Park, N.C., USA). Catheter patency was checked periodically by administering 500  $\mu$ g methohexital through the catheter. If patent, loss of consciousness occurred within 1–2 s.

### Apparatus

All experimental sessions were conducted in sound-attenuated chambers and were controlled by an IBM-compatible computer through an interface (Med Associates Inc., St Albans, Vt., USA). The operant chamber (21 cm $\times$ 21 cm $\times$ 28 cm) contained a response lever 6.8 cm above the floor and 1.1 cm from the rear wall and a light located 4.0 cm above the response lever. Each sound-attenuated chamber contained a house light, tone generator and a ventilator fan. The fluid swivel and catheter were connected to a 20 ml syringe on an infusion pump located outside of the sound-attenuated enclosure through a 20 ga Luer hub and a 22 ga male connector.

### Heroin self-administration

Lever presses were engendered and maintained in animals ( $n=42$ ) with infusions of 18  $\mu$ g heroin (90  $\mu$ g/ml) in a volume of 0.2 ml over 5.6 s as described previously (Martin et al. 1998). Following 5–7 days of recovery from surgery, animals were trained to self-administer heroin under a fixed-ratio 1 schedule of reinforcement. A light above the lever was illuminated to indicate drug availability and, upon completion of the ratio requirement, the lever light was darkened and an infusion of heroin was delivered. A 30-s time-out period followed that was signaled by the operation of the house light and tone. Sessions were conducted on Monday through Friday and were 4 h in duration. Responding was considered stable when the number of infusions delivered for each of 5 successive days did

not vary by more than 10% of the mean. When stable responding was established, the ratio requirement was increased from 1 to 10 across experimental sessions. Saline was substituted for all doses of heroin within a session following 5 days of stable responding at FR10. The dose-effect curve was generated by substituting 0.3, 0.9, 1.8, 5.4, 9 or 30  $\mu\text{g}/\text{infusion}$  of heroin for the training dose on Tuesdays or Thursdays provided the number of infusions administered during the preceding session did not vary by more than 10% from the mean of previous sessions. Duplicate determinations were made for saline and each dose of heroin.

A separate group of animals ( $n=10$ ) was trained to self-administer four doses of heroin made available during a single session using previously published procedures (Martin et al. 1996). The doses used for training were 5.4, 9, 18 and 30  $\mu\text{g}/\text{infusion}$ . Each dose of heroin was made available for self-administration for 1 h using an FR10 schedule and each hourly component was separated by a 20-min blackout period. The order of dose presentation was randomized for each session. As with the other procedure, saline was substituted for heroin at least twice on either Tuesdays or Thursdays.

### $\beta$ -Funtaltrexamine administration

$\beta$ -FNA (0.25, 1.25 or 2.5 nmol) or vehicle [0.9% saline (w/v) pH 7.4] was administered in a volume of 1.0  $\mu\text{l}$  at a flow rate of 0.2  $\mu\text{l}/\text{min}$ . Internal injection cannulae were connected to Hamilton microsyringes using polyethylene tubing (Plastics One) and infusions were delivered using a microsyringe infusion pump (KDS Scientific, Boston, Mass., USA). Injection cannulae were left in place for 15 min after the injection to allow for pressure equilibration. Beginning 24 h after NAcc injection, each animal was allowed to self-administer heroin for up to 30 days following  $\beta$ -FNA or vehicle administration. Each animal was given only one intra-NAcc injection.

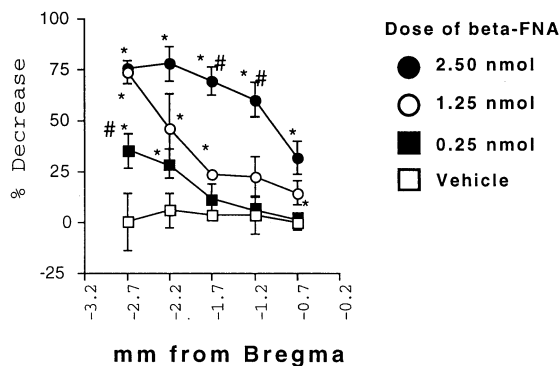
### Effect of $\beta$ -FNA on [ $^3\text{H}$ ]DAMGO and [ $^3\text{H}$ ]DPDPE autoradiographic binding in the NAcc

The quantitative autoradiography procedures used to determine mu or delta opioid receptor density have been described previously (Martin et al. 1993). Serial sections (20  $\mu\text{m}$ ) were thaw-mounted onto gelatin-coated slides at  $-20^\circ\text{C}$  from five different levels of the NAcc, corresponding to  $-2.7$ ,  $-2.2$ ,  $-1.7$ ,  $-1.2$  and  $-0.7$  mm rostral relative to bregma identified according to the atlas of Paxinos and Watson (1986). Sections were taken from animals 24 h after administration of  $\beta$ -FNA (0.25, 1.25 or 2.5 nmol) or vehicle ( $n=8/\text{group}$ ) unilaterally into the rostral NAcc. The contralateral side served as control for each animal. Briefly, sections were preincubated for 20 min in buffer (50 mM TRIS HCl, pH 7.4) at  $25^\circ\text{C}$ , followed by a 2.5-h incubation in either 2 nM [ $^3\text{H}$ ]DAMGO or 4 nM [ $^3\text{H}$ ]DPDPE for mu or delta-opioid receptors, respectively. These concentrations correspond to a saturating level for mu opioid receptors (DAMGO) or the approximate  $K_d$  concentration for delta opioid receptors (DPDPE) under these conditions (Martin et al. 1993). Non-specific binding was assessed in the presence of 1  $\mu\text{M}$  naloxone in adjacent sections. Sections were then washed for 2 min in ice-cold buffer, dipped in ice-cold distilled water for 10 s and rapidly dried under a stream of dry, cool air. Sections were apposed to  $^3\text{H}$ -sensitive film (Hyperfilm- $^3\text{H}$ , Amersham, Arlington Heights, Ill., USA) with calibrated standards ( $^3\text{H}$ -microscales; Amersham) for 9 weeks.

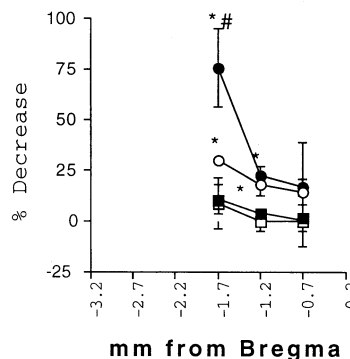
### Data analysis

Behavioral data were analyzed for dose-responsiveness with respect to heroin and  $\beta$ -FNA dose using ANOVA. The time-course of  $\beta$ -FNA's effects were analyzed using repeated measures ANOVA and post-hoc analyses were performed using Bonferroni-Dunn  $t$ -test for multiple comparisons to a control, with each animal's pre-treatment self-administration data serving as control. Within-ses-

## A. Rostral Pole and Shell



## B. Core



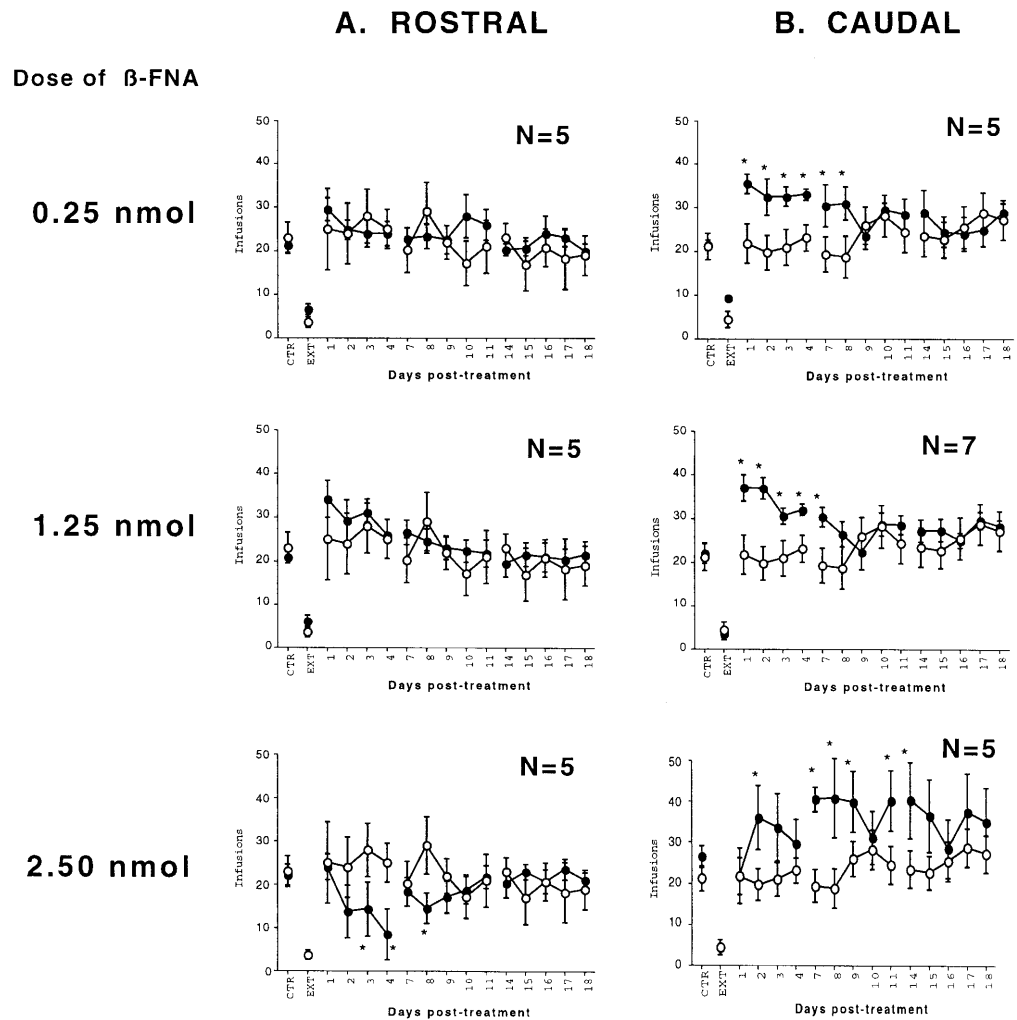
**Fig. 1A, B** Effect of  $\beta$ -FNA on [ $^3\text{H}$ ]DAMGO binding. Data are mean (SEM) expressed as percent decrease in  $\beta$ -FNA-treated side compared to the contralateral side for each subregion of the NAcc. **A** The rostral pole is defined as the NAcc at the levels of  $-2.7$  and  $-2.2$  mm relative to bregma. **B** The shell and core are delineated at levels  $-1.7$ ,  $-1.2$  and  $-0.7$  mm relative to bregma according to the atlas of Paxinos and Watson (1986). The injection sites for these animals were all within the NAcc at the level of  $-2.2$  mm relative to bregma. \*Significantly different from zero,  $P=0.05$ . #Significantly different from 1.25 nmol dose,  $P=0.05$ .  $n=8$  for each treatment group

sion dose-response data were analyzed using two-factor ANOVA with heroin dose and time following  $\beta$ -FNA or saline treatment serving as the independent variables. Binding data were analyzed using a two-factor ANOVA with coronal level and  $\beta$ -FNA dose as the independent variables and percent loss of either [ $^3\text{H}$ ]DAMGO or [ $^3\text{H}$ ]DPDPE binding density compared to the contralateral side as the dependent measure.

### Drugs and chemicals

Heroin hydrochloride and  $\beta$ -funtaltrexamine hydrochloride were obtained from the Drug Supply Program of the National Institute on Drug Abuse of the National Institutes of Health. Heroin hydrochloride was dissolved in 0.9% (w/v) saline, pH 7.4 with heparin (1.7 IU/ml) and doses are expressed in terms of the free base.  $\beta$ -Funtaltrexamine hydrochloride (molecular weight 490) was dissolved in 0.9% (w/v) saline, pH 7.4. [ $^3\text{H}$ ]DAMGO (55.0 Ci/mmol) and [ $^3\text{H}$ ]DPDPE (44.0 Ci/mmol) were purchased from New England

**Fig. 2A, B** Effect of  $\beta$ -FNA on heroin self-administration. Data are mean (SEM) for number of infusions of 18  $\mu$ g of heroin administered during the 4-h session. *Open symbols* represent data from animals given vehicle into the rostral (A) or caudal (B) NAcc, whereas *closed symbols* represent data from animals administered the respective doses of  $\beta$ -FNA. Mean number of infusions for the five sessions prior to vehicle or  $\beta$ -FNA administration are indicated by *CTR* and the number of infusions administered during saline substitution for heroin are indicated by *EXT*. \*Significantly different from CTR,  $P=0.05$



Nuclear (Boston, Mass., USA). Hyperfilm-H<sup>3</sup> and <sup>3</sup>H-microscales were purchased from Amersham. Sodium pentobarbital (Nembutal) was purchased from Abbott Laboratories (North Chicago, Ill., USA) in a vehicle of 10:40:50 (v/v) ethanol:propylene glycol:water. Methohexital (Brevital) was purchased from Eli Lilly Co. (Indianapolis, Ind., USA) and dissolved in sterile water. Antibiotic powder (Polysporin) was purchased from Wellcome-Glaxo Inc. and heparin was purchased from Elkins-Sinn Co. (Cherry Hill, N.J., USA).

## Results

### Biochemical and anatomical specificity of intra-accumbens injections of $\beta$ -FNA

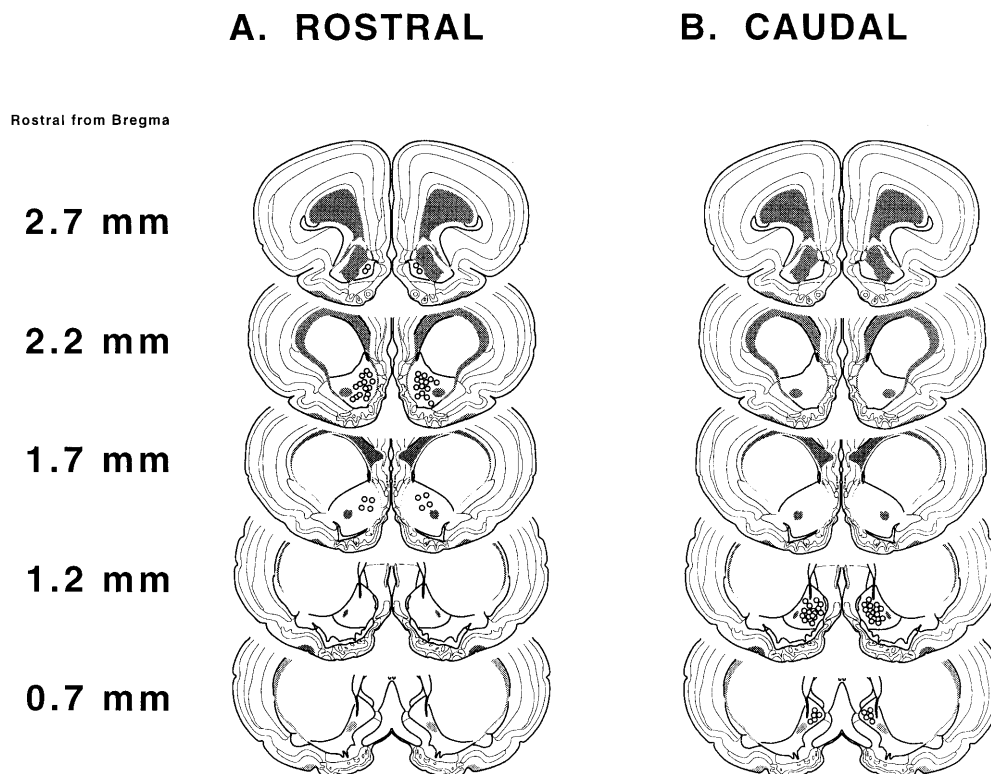
$\beta$ -FNA attenuated [<sup>3</sup>H]DAMGO binding in the NAcc in a dose-dependent manner in the rostral pole [ $F(3,56)=933$ ,  $P=0.0001$ ], shell [ $F(3,84)=1250$ ,  $P=0.0001$ ] and core [ $F(3,84)=390$ ,  $P=0.0001$ ], resulting in a maximal decrease of 80% in mu opioid receptor density (Fig. 1A). The effect of  $\beta$ -FNA was also dependent upon the distance from the guide cannula in all three regions: [ $F(1,56)=10.5$ ,  $P=0.002$ ;  $F(2,84)=192$ ,  $P=0.0001$ ;  $F(2,84)=127$ ,  $P=0.0001$ ] for the rostral pole, shell and core, respectively. There was a significant interaction between distance from the cannu-

la and the dose of  $\beta$ -FNA as well in the rostral pole [ $F(3,56)=42.8$ ,  $P=0.0001$ ], shell [ $F(6,84)=81.1$ ,  $P=0.0001$ ] and core [ $F(6,84)=64.6$ ,  $P=0.0001$ ]. The highest dose of  $\beta$ -FNA produced decrements up to 1.5 mm from the site of administration and the lowest dose affected binding only within 0.5 mm from the injection site (Fig. 1A,B). [<sup>3</sup>H]DPDPE binding density was significantly decreased ( $25\pm 5.6\%$  of control) only by the highest dose of  $\beta$ -FNA administered (2.5 nmol) and all effects were at the site of injection with no decrement in binding occurring in any other sections (data not shown). These effects of  $\beta$ -FNA on [<sup>3</sup>H]DAMGO binding were localized to the NAcc, as the binding in adjacent regions such as the caudate putamen ( $96\pm 8.0\%$  control), olfactory tubercles ( $105\pm 9\%$  control) and prefrontal cortex ( $92\pm 8\%$  control) were not significantly different from control values ( $\alpha=0.05$ ).

### Effect of administration of $\beta$ -FNA into the rostral NAcc on heroin self-administration

Heroin maintained robust responding ( $21.8\pm 1.0$  infusions/4 h) in the group of animals used for injections into the rostral pole of the NAcc and substitution of saline for

**Fig. 3** Injection sites for administration of beta-FNA into the rostral (**A**) or caudal (**B**) NAcc. The tip of the injection cannula is represented by *open symbols* for the animals used to generate the data shown in Fig. 2



heroin resulted in a decrease in responding ( $5.1 \pm 0.6$  infusions/4 h) in these animals [ $F(1,38)=237.2$ ,  $P < 0.0001$ ]. Injection of vehicle into the rostral portion of the NAcc did not significantly alter the number of infusions of heroin administered at any time point [ $F(14,84)=0.606$ ,  $P=0.9$ ] (Fig. 2A). The effects of  $\beta$ -FNA were not dose-responsive [ $F(3,29)=0.427$ ,  $P=0.7$ ] 24 h following injection into the rostral pole and there was no significant main effect of  $\beta$ -FNA treatment [ $F(1,30)=0.159$ ,  $P=0.7$ ] (Fig. 2A). Histological analysis demonstrated that the injection sites were within the NAcc between 2.7 and 1.7 mm rostral relative to bregma for these animals (Fig. 3A).

#### Effect of administration of $\beta$ -FNA into the caudal NAcc on heroin self-administration

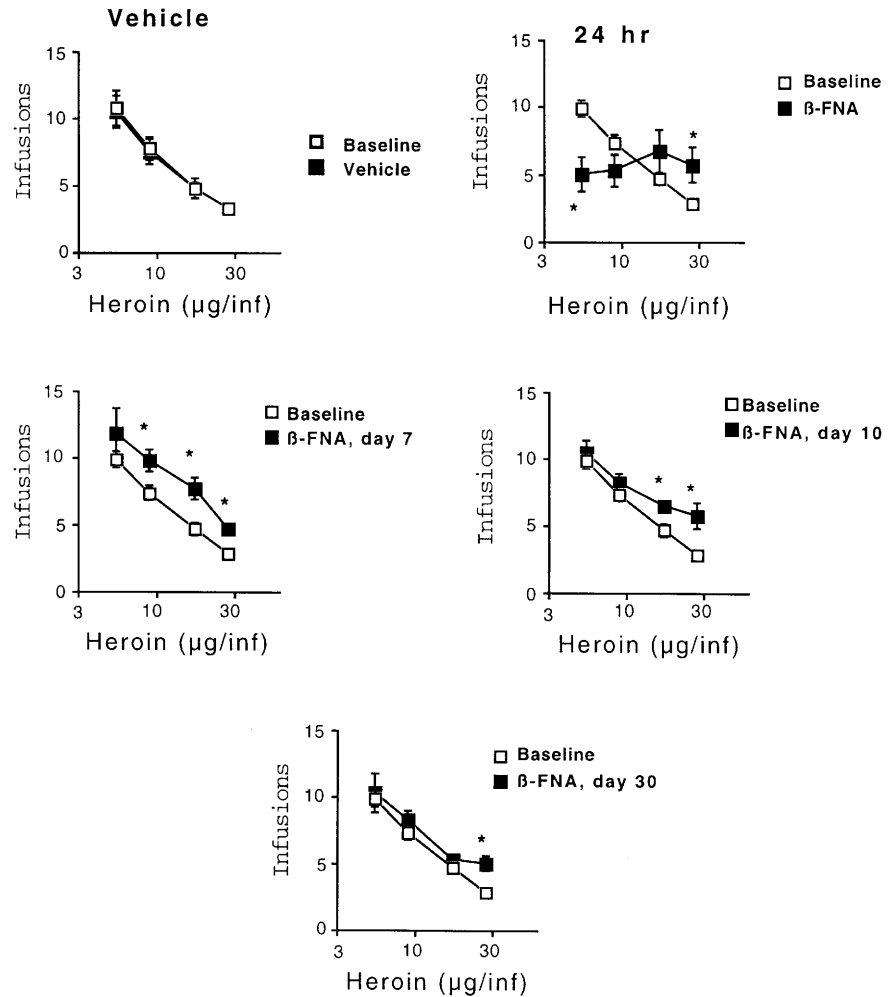
Heroin maintained robust responding ( $22.4 \pm 1.2$  infusions/4 h) in the group of animals used for injections into the caudal NAcc and substitution of saline for heroin resulted in a decrease in responding ( $4.7 \pm 0.6$  infusions/4 h) in these animals [ $F(1,42)=186.7$ ,  $P < 0.0001$ ]. Injection of vehicle into the caudal NAcc did not significantly alter the number of infusions of heroin administered at any time following injection [ $F(14,84)=0.606$ ,  $P=0.8532$ ] (Fig. 2B). Unlike the data obtained following administration into the rostral pole, there was a significant main effect of  $\beta$ -FNA treatment [ $F(1,31)=6.65$ ,  $P=0.015$ ] and the effects of  $\beta$ -FNA were dose-dependent [ $F(3,31)=2.92$ ,  $P=0.049$ ] 24 h after administration into the caudal portion of the NAcc. Injection of 0.25 nmol

$\beta$ -FNA into the caudal NAcc resulted in a significant increase in the number of infusions of heroin administered for up to 7 days compared to vehicle-injected controls [ $F(14,45)=2.613$ ,  $P=0.0075$ ] (Fig. 2B). Injection of 1.25 nmol  $\beta$ -FNA into the caudal NAcc also produced an increase in the number of infusions of heroin administered for up to 7 days [ $F(14,56)=3.85$ ,  $P < 0.0001$ ] (Fig. 2). Injection of 2.5 nmol  $\beta$ -FNA into the caudal NAcc increased the number of infusions of heroin on days 2–15 following administration [ $F(14,45)=1.506$ ,  $P=0.049$ ] (Fig. 2B). Histological analysis revealed that the injection sites were within 1.2–0.7 mm rostral relative to bregma and within the NAcc for these animals (Fig. 3B).

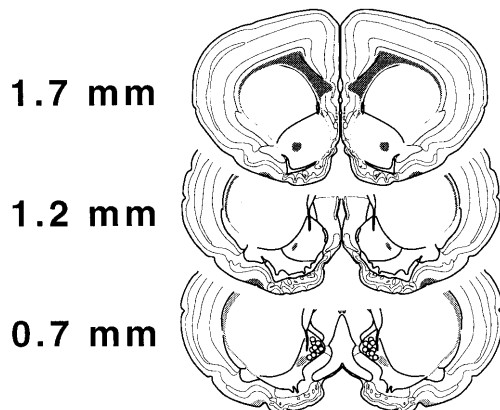
#### Effect of $\beta$ -FNA injection into the caudal NAcc on the dose-effect curve for heroin self-administration

The effects of injection of  $\beta$ -FNA into the caudal portion of the NAcc on the dose-effect curve for heroin self-administration were characterized. Injection of saline into the caudal portion of the NAcc had no significant effect on the self-administration of any dose of heroin at any time point using the within-session dose-response procedure [ $F(72,268)=0.576$ ,  $P=0.997$ ] (Fig. 4). Administration of  $\beta$ -FNA into the caudal portion of the NAcc resulted in an apparent flattening of the dose-effect curve for heroin self-administration (Fig. 4). The effect of  $\beta$ -FNA was dependent upon both the time after injection and the dose of heroin [ $F(72,359)=1.65$ ,  $P=0.0017$ ]. Injection of 2.5 nmol of  $\beta$ -FNA significantly reduced the

**Fig. 4** Effect of administration of 2.5 nmol  $\beta$ -FNA into the caudal NAcc on the dose-effect curve for heroin self-administration. The mean (SEM) numbers of infusions of four doses of heroin are shown 24 h after vehicle injection or at various time points following administration of 2.5 nmol  $\beta$ -FNA into the caudal NAcc. Baseline data represent the mean (SEM) for the five sessions preceding vehicle or  $\beta$ -FNA administration. \*Significantly different from baseline,  $P=0.05$ .  $n=5$ /group



Rostral from Bregma



**Fig. 5** Injection sites for caudal NAcc. *Open symbols* represent the placement of the injection cannula tip for animals used to generate the data shown in Fig. 4

number of infusions of 5.4  $\mu$ g and 9  $\mu$ g of heroin administered for up to 4 days after administration, with the number of infusions returning to baseline levels at 7 and 10 days, respectively. Administration of 2.5 nmol  $\beta$ -FNA into the caudal NAcc resulted in a significant increase in

the number of infusions of 18 and 30  $\mu$ g of heroin administered for up to 17 days. The number of infusions administered of 18  $\mu$ g heroin returned to baseline levels after 18 days and was not significantly different from baseline for up to 30 days thereafter ( $\alpha=0.05$ ). Histological analysis demonstrated that the injection sites were at the level of 0.7 mm rostral relative to bregma and within the NAcc for these animals (Fig. 5).

## Discussion

The major finding of this study is that mu-opioid receptors in the caudal portion of the nucleus accumbens appear to have a greater role in the processes underlying self-administration of heroin than those in the rostral pole. This is the first investigation that suggests a delineation between the rostral pole and the caudal NAcc exists at the level of the whole animal with respect to opioid receptor function. Although salient differences have been found with respect to functional neuroanatomy along the rostrocaudal axis of the accumbens, pharmacological data to support the significance of these differences has been lacking. These data clearly identify the caudal NAcc and its projections as prospective regions

for future pharmacological and electrophysiological investigations of the reinforcing effects of opioids. Further delineation at the level of the core and shell regions within the caudal accumbens with respect to heroin self-administration would also likely yield interesting findings.

The shift in the heroin dose-effect curve following  $\beta$ -FNA administration appears to be downward and to the right initially, followed by an apparent upward shift as mu-opioid receptor density recovers. The downward and rightward shift is what would be expected from a non-competitive antagonist such as an alkylating agent at doses sufficient to deplete any spare receptor population. The upward shift likely occurs as new mu-opioid receptors are synthesized to replace their alkylated counterparts and reach a level sufficient to produce a maximal effect, but not at the full homeostatic receptor density, therefore requiring a greater concentration of drug (i.e. greater numbers of infusions/unit time) to produce similar percent receptor occupancy levels and thereby similar reinforcing effects. The relatively long time course of  $\beta$ -FNA's actions on heroin self-administration is similar to that reported previously following ICV injection (Martin et al. 1998) and suggests that a depot of drug is produced, perhaps a glutathione conjugate that can be activated into a receptor-alkylating compound in vivo (Larson et al. 1993). The effects of  $\beta$ -FNA in the present study are unlikely to be caused by diffusion into the cerebral ventricles, as doses of 10 nmol ICV or higher are necessary to produce even modest changes in heroin self-administration (Martin et al. 1998) and effects were produced in the present study by doses as low as 0.25 nmol injected directly into the NAcc. It is possible that a portion of this dose entered the cerebral ventricles; however, the majority of the behavioral effect was probably due to inhibition of mu-opioid receptors within the caudal NAcc.

The nucleus accumbens is a complex structure and the intricacies of its cytoarchitecture and functional neuroanatomy have been appreciated for some time. A complete review of the neuroanatomy of the nucleus accumbens is provided elsewhere (Zahm and Brog 1992; Zahm 2000). The rostral pole of the accumbens comprises approximately the rostral quarter of the structure and has features of both the core and shell subregions that are clearly delineated in the more caudal areas (Zahm and Brog 1992). The primary afferents to the rostral pole come from the cortex and striatum and the primary efferent fibers project to the substantia nigra and pallidum. More caudally, the primary afferents come from the ventral tegmental area and dorsal striatum as well as from numerous cortical areas whereas the primary efferent fibers project to the thalamus and amygdala. The present data indicate that heroin self-administration is more sensitive to disruption of heroin's effects on the thalamus and amygdala mediated through the accumbens rather than those effects on the nigra and pallidum that would be mediated through the rostral pole. This does not preclude the pallidum and nigra from being important in

heroin self-administration, but does suggest that the influence of heroin's effects on the thalamus and amygdala mediated through mu-opioid receptors in the accumbens deserve some attention.

It is difficult to postulate which of the myriad of substrates involving input and output regions of the caudal accumbens provide for the differential effects of  $\beta$ -FNA treatment in the rostral pole compared to the caudal areas, however it is likely that the underlying mechanism is the result of action at several relevant sites along these pathways. Rostrocaudal gradients are known to exist for both mu-opioid receptor binding (Mansour et al. 1988) and proenkephalin gene expression within the accumbens (Rogard et al. 1993). Enkephalinergic patches are also more numerous in the caudal region of the accumbens compared to the rostral pole, possibly indicating an increased role of endogenous opioids in the neurochemical modulation of this region compared to the rostral pole (Rogard et al. 1993). Regions likely to be influenced by opioid receptor activation in the caudal NAcc include the medial thalamus, amygdala, parabrachial nucleus and solitary complex of the brainstem (Zahm and Brog 1992). The caudal and medial aspects of the NAcc display neuroanatomical characteristics of the extended amygdala, with the more rostral aspects of the accumbens resembling striatopallidal circuitry exclusively (Alheid and Heimer 1988; Heimer et al. 1991; Alheid et al. 1995). Output regions of the rostral pole include the pallidum and VTA, although these regions also receive input from more caudal regions of the accumbens.

The present data clearly identify  $\beta$ -FNA as a useful tool for investigating the functional neuroanatomy of discrete populations of mu-opioid receptors. The key features of  $\beta$ -FNA are its irreversibility, hydrophilicity and receptor selectivity. The irreversible nature of  $\beta$ -FNA's effects is well-documented and the site of electrophilic addition to the mu-opioid receptor has been identified as Lys233 (Chen et al. 1996). Quantification of the anatomical and receptor subtype specificity of  $\beta$ -FNA following ICV administration is only possible because its effects on receptor binding are irreversible. Hydrophilicity is important because it allows  $\beta$ -FNA to be administered in a vehicle of low toxicity (physiological saline at neutral pH) at doses that significantly decrease mu-opioid receptor density. The hydrophilic nature of  $\beta$ -FNA also undoubtedly contributes to the anatomically discrete localization of receptor alkylation as lipophilic compounds are likely to spread much farther from the site of injection. The selectivity of  $\beta$ -FNA's irreversible effects on mu-opioid binding is obviously important for discriminating between effects on mu- and delta-opioid receptors.  $\beta$ -FNA was clearly shown to be mu-opioid selective at doses up to 1.25 nmol which significantly decreased mu-opioid binding density and produced significant behavioral effects on heroin self-administration when injected into the caudal portion of the NAcc.  $\beta$ -FNA could obviously be used for similar studies with practically any brain region and functional

correlate of interest. Hopefully, such future studies will be useful in mapping the functional neuroanatomy of the mu-opioid receptor system.

In summary, these data suggest that the caudal NAcc is a region critical for mu-opioid receptor involvement in heroin self-administration. Future studies should prove useful in determining the plasticity of this region in mediating the self-administration of opioids. Examination of the effects of chronic heroin exposure on subregions of the caudal NAcc and their respective output sites, such as the amygdala and thalamus, may also yield information regarding the plasticity of the NAcc and the relevance of neuronal changes in these regions to the reinforcing effects of heroin.

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